Production of Fusion Proteins

The components of the fusion protein can be linked to each other, preferably via a linker sequence. The linker sequence should separate the first and second members of the fusion protein by a distance sufficient to ensure that each member properly folds into its secondary and tertiary structures. Preferred linker sequences (1) should adopt a flexible extended conformation, (2) should not exhibit a propensity for developing an ordered secondary structure which could interact with the functional first and second component, and (3) should have minimal hydrophobic or charged character, which could promote interaction with the functional protein domains. Typical surface amino acids in flexible protein regions include Gly, Asn and Ser. Permutations of amino acid sequences containing Gly, Asn and Ser would be expected to satisfy the above criteria for a linker sequence. Other near neutral amino acids, such as Thr and Ala, can also be used in the linker sequence.

A linker sequence length of 20 amino acids can be used to provide a suitable separation of functional protein domains, although longer or shorter linker sequences may also be used. The length of the linker sequence separating the first and second components can be from 5 to 500 amino acids in length, or more preferably from 5 to 100 amino acids in length. Preferably, the linker sequence is from about 5-30 amino acids in length. In preferred embodiments, the linker sequence is from about 5 to about 20 amino acids, and is advantageously from about 10 to about 20 amino acids. Amino acid sequences linkers of the first and second member include, but are not limited to, (SerGly4)y (SEQ ID NO 6) wherein y is greater than or equal to 8, or $Gly_4SerGly_5Ser$ (SEQ ID NO 7). A preferred linker sequence has the formula $(SerGly_4)_4$ (SEQ ID NO 8). Another preferred linker has the sequence $((Ser\text{-}Ser\text{-}Ser\text{-}Ser\text{-}Gly)_3\text{-}Ser\text{-}Pro)$ (SEQ ID NO 9).

The first and second components can be directly fused without a linker sequence. Linker sequences are unnecessary where the proteins being fused have non-essential N-or C-terminal amino acid regions which can be used to separate the functional domains and

1 cycle	94°	60 sec
5 cylces	94°C	30 sec
	58°C	45 sec
	74°C	45 sec
30 cycles	94°C	30 sec
	55°C	30sec
	74°C	30 sec

Primer sets:

GBC 332 and GBC 386, amplicon is 206 bp

GBC 332: TGTGCTCCTCTCCATGCTGG (SEQ ID NO: 10)

GBC 386 TGGTCTGGGGTGACACATGT (SEQ ID NO: 11)

Southern blot analysis of transgenic founders:

Genomic DNA ((24 pg total, 8 pg/lane) from each founder mouse positive for the insulator PCR was digested to completion with the restriction enzyme EcoRI. Digested DNAs were electrophoresed in triplicate and transferred to nylon membranes according to standard methods (Maniatis et al., 1982). Probes specific for each expression cassette were isolated from the VK (LC1O in pSP72, 72 bp probe), ProL (pMF141-4 in pSP72 345, bp probe), and fd-CPB (pMF213-20 in pSP72, 1861 bp probe) plasmids (provided by Michael